REMARKS

With this response, claims 93-116 and new claims 117-124 are pending in the present application and currently under examination. For the Examiner's convenience, Applicants address the rejections in the order presented in the July 17, 2001 Office Action. Appendix A provides the Version with Markings to Show Changes Made. All pending claims are provided in Appendix B for the Examiner's convenience.

Applicants thank the Examiner for returning to Applicants an initiated copy of the PTO form 1449 received by the PTO on August 4, 2000. However, Applicants respectfully point out that the Examiner omitted to initial reference AF (Disis *et al.*) on page 2 of the PTO form 1449. As Applicants believe that this reference was properly presented for consideration, Applicants request that the Examiner provide Applicants with a copy of the PTO form 1449 with reference AF initialed as considered.

Status of the specification

The specification was objected to for use of the registered trademark HERCEPTIN®. Applicants have amended the specification to capitalize the trademark, and to recite generic terminology ("Her-2/neu antibody"). Applicants therefore respectfully request that the objection be withdrawn.

Status of the claims

Claims 93 and 103 were amended to delete the recitation of 80% identity to the reference sequence identifiers, and to instead recite hybridization under specified hybridization and wash conditions to SEQ ID NO:6 or SEQ ID NO:7. This amendment adds no new matter. Support for this amendment can be found, e.g., in the specification on page 11, line 31 to page 12, line 5.

Claims 94, 95, 96, 104, 105, and 106 were amended to delete the term "fused" and to recite the term "linked." This amendment adds no new matter. Support for this amendment can be found, e.g., in the specification on page 8, lines 7-13.

Claims 99 and 109 were amended to delete the term "pharmaceutically" acceptable carrier, and to recite the term "physiologically" acceptable carrier. This amendment adds no new matter. Support for this amendment can be found, e.g., in the specification on page 48, line 11.

New claims 117-120 and 122-123 were added these claims add no new matter. Support for these claims can be found, e.g., in the claims as originally filed.

New claims 121 and 124 were added. These claims add no new matter. Support for these claims can be found, e.g., in the specification on page 15, line 4.

Rejection under 35 U.S.C. § 112, second paragraph: indefiniteness

Claim 116 was rejected for reciting apparent trademarks. To expedite prosecution, these terms have been deleted. Applicants therefore respectfully request that the rejection be withdrawn.

Rejection under 35 U.S.C. § 112, first paragraph: written description

Claims 93-116 were rejected as allegedly containing subject matter that was not described in the specification as originally filed. In the Office Action, the Examiner observed that the purpose of the written description requirement is to convey to one of skill in the art that the inventor was in possession of the invention as of the filing date. The rejection then stated that the claims are broadly drawn to a polynucleotide of any size comprising a sequence that is at least 80% homologous to SEQ ID NO:6 or SEQ ID NO:7 or a fusion of SEQ ID NO:3 to SEQ ID NO:4 or a fusion of SEQ ID NO:4 to SEQ ID NO:5, as well as corresponding vectors, host cells, and methods of making a polypeptide.

Applicants respectfully traverse this rejection. Applicants first note that the claims have been amended to recite nucleic acid sequences that hybridize under specified stringent conditions to the complement of sequences encoding SEQ ID NO:6 or SEQ ID NO:7, which are Her-2/neu fusion proteins comprising an extracellular fused to a phosphorylation domain.

The claims fully comply with the requirements for written description of a chemical genus as set forth in *University of California v. Eli Lilly & Co.*, 43 USPQ2d 1398 (Fed. Cir. 1997). As described by the Federal Circuit in *Lilly*, "[a] description of a genus of cDNAs may be achieved by means of . . . a recitation of structural features common to the members of the genus . . . ." *Lilly*, 43 USPQ2d at 1406. Furthermore, the court in *Fiers v. Revel* stated that an adequate written description "requires a precise definition, such as by structure, formula, chemical name, or physical properties." *Fiers v. Revel*, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993). The claims set forth both functional elements as well as structural elements, i.e., hybridization conditions and reference sequences to which members of the claimed genus hybridize. Therefore, the claimed sequences are thereby defined via shared physical and structural properties.

As described above, the present invention relates to a fusion protein comprising a Her-2/neu extracellular domain fused to a Her-2/neu phosphorylation domain. The genus of Her-2/neu nucleic acids and the fusion proteins that they encode are claimed by reference to shared structural features, i.e., structural features of nucleic acid sequences that encode a Her-2/neu fusion protein comprising an extracellular domain and a phosphorylation domain (SEQ ID NO:6 or 7). The claims also provide specific hybridization conditions in which the claimed genus of Her-2/neu nucleic acids hybridize to nucleic acids encoding the reference conserved sequences (e.g., the "hybridization reaction is incubated in a solution comprising 5x SSC at a temperature of 50-65°C and washed in a solution comprising 0.2x SSC and 0.1% SDS at a temperature of 65°C").

The ability of a particular nucleic acid to hybridize under *given conditions* to a reference sequence is a physical/structural property of the nucleic acid, because it relies upon the nucleotide sequence of the molecule (*see, e.g.*, Sambrook, *Molecular Cloning: A Laboratory Manual*, pp. 9.47-9.51 (2nd ed. 1989); *see also* Stryer, *Biochemistry*, pp. 573 (2nd ed. 1975)). As described in Stryer, the transition between hybridization and melting of complementary nucleic acid strands is abrupt and largely sequence dependent. When the temperature of hybridization is provided, one of skill in the art would be able to predict

whether or not a given sequence would hybridize to a reference sequence (*see, e.g.*, equations provided in Sambrook, *supra*).

In the present application, Applicants have provided both reference nucleotide sequences, as well as hybridization conditions. As required by the standard set forth in *University of California v. Eli Lilly*, these structural features are common to all of the members of the claimed genus of Her-2/neu nucleic acids encoding fusion proteins. The conserved sequences encoding structural features of the genus, and the given conditions under which the claimed genus would hybridize to such reference sequences "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed" (*see* Office Action, page 5, *quoting Vas-Cath Inc. v. Mahurkar*, 19 USPQ2d 111, 1116 (Fed. Cir. 1991)). The specification thus appropriately describes the claimed Her-2/neu nucleic acid and fusion protein genus using structural/physical features, as required by the court in *University of California v. Eli Lilly*. As such, Applicants respectfully request that the Examiner withdraw the rejection.

Rejection under 35 U.S.C. § 112, second paragraph: enablement

Claims 99-102 and 109-112 were rejected as allegedly lacking enablement for reciting "pharmaceutical" compositions. Applicants have amended the claims to delete the term "pharmaceutical" so that the claims now more broadly recite "compositions." The rejection stated that when a composition is limited by a particular use, enablement of the claim should be based on that limitation. Applicants have therefore amended the claim to remove the limitation "pharmaceutical." The claim now reads on all compositions comprising fusion proteins of the invention. Such compositions are useful for numerous applications. MPEP § 2164.01(c) describes the enablement standard for compound and composition claims as follows:

> [W]hen a compound or composition claim is not limited by a recited use, any enabled use that would reasonable correlate with the entire scope of that claim is sufficient to preclude a rejection for nonenablement based on how to use. . . . In other words, if any use is enabled when multiple uses are disclosed, the application is enabling for the claimed invention."

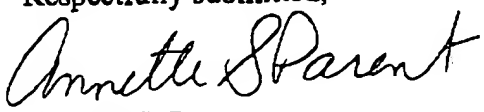
Applicants therefore respectfully request withdrawal of the rejection.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Please amend the specification at page 6, lines 14-17, as follows:

Figure 17 illustrates the results of an ELISA assay for [Herceptin] HERCEPTIN® Her-2/neu antibody binding to different ECD-PD fusion proteins produced either in mammalian cells or in *E. coli*. The fusion proteins produced in *E. coli* are in frame with a C- or N-terminal 6 x histidine tag (noted C-His tag and N-His tag, respectively).

Please amend the specification at page 45, line 31 to page 46, line 2, as follows:

Examples of available suitable antibodies to the fusion proteins of the invention include, but are not limited to, the 8029K rabbit polyclonal antibody, the mouse monoclonal c-neu-3 antibody (Calbiochem), and the mouse monoclonal [Herceptin] HERCEPTIN® Her-2/neu antibody (U.S. Patent 5,677,171). The monoclonal c-neu-3 antibody recognizes a sequential epitope in the PD domain which is deleted (1242-1255 aa) in the ECD-ΔPD construct. The [Herceptin] HERCEPTIN® Her-2/neu antibody binds to a conformational epitope in the ECD domain.

Please amend the specification on page 73, lines 21-25, as follows:

The *E. coli* produced hECD-PD.$C_T$his was then purified on a monoQ column, and refolded in 20 mM Tris-HCL (pH 8.0) buffer. The refolded protein was tested and found to be positive for [Herceptin] HERCEPTIN® Her-2/neu antibody binding by Western blot and ELISA (Fig. 17). The [Herceptin] HERCEPTIN® Her-2/neu antibody binding activity was, however, lost later on, probably due to denaturation of the protein.

Please amend the specification on page 74, lines 3-6 as follows:

The *E. coli* derived unpurified $N_T$his-hECD-PD fusion protein was recognized by the mouse c-neu-3 antibody and by a rabbit anti-ECD antibody. Following purification, the *E. coli* derived $N_T$his-hECD-PD was recognized by [Herceptin] HERCEPTIN® Her-2/neu antibody both in Western blots and in ELISA assays (Fig. 17).

Please amend the specification on page 76, lines 28-33 as follows:

In the cell-free supernatants, secretion of full-length ECD-PD recombinant protein was very weak and only detected on Western Blots using the c-neu-3 mouse antibody (Calbiochem). Secretion and accumulation (maximum after 72 hours) of a + 70kDa protein was visible on Silver stained SDS-PAGE and detected on Western blot under non-reducing conditions with [Herceptin] HERCEPTIN® Her-2/neu mouse antibody. This protein was not detected using the mouse c-neu-3 antibody or a mouse anti-histidine antibody (QIAGEN).

Please amend the specification on page 78, lines 4-24, as follows:

The MSX transfectant clones were transferred 3-5 weeks after transfection into 24-well plates and the culture supernatants were harvested. Expression of the ECD-PD or ECD-ΔPD fusion proteins was tested by Western blot analysis using [Herceptin] HERCEPTIN® Her-2/neu antibody under non reducing conditions. Expression of the ECD-PD fusion protein was detected in 18 out of 52 clones tested, while 13 out of 47 clones tested were positive for ECD-ΔPD expression. The selected clones expressing the fusion proteins were then readapted to suspension serum-free conditions. Based on the level of expression, growth and viability, 5 clones carrying the ECD-PD construct and 3 clones carrying the ECD-ΔPD construct were further evaluated and characterized. For the ECD-PD construct, clone 560 F3 showed the highest expression level.

Expression was evaluated at 33°C in the presence or absence of sodium butyrate (2 mM) and of DMSO (2 %). Some of the clones were inducible by NaB or DMSO. Expression in CHO-K1 cells of ECD-PD and ECD-ΔPD was analyzed by Western blots and SDS-PAGE followed by either Silver or Coomassie staining. The [Herceptin] HERCEPTIN® Her-2/neu antibody and the c-neu-3 mouse monoclonal antibodies, as well as the 8029K rabbit polyclonal antibody were used for Western blot analysis. Analysis of the culture supernatants from ECD-PD and ECD-ΔPD clones showed a band in Coomassie/Silver stained gels at 150 kDa and at 98 kDa, respectively. The same bands were revealed by [Herceptin] HERCEPTIN® Her-2/neu antibody and by the 8029K polyclonal antiserum, as well as by the c-neu-3 antibody for ECD-PD only (Fig. 18). The CHO-expressed HER-2/neu fusion proteins are recognized by the [Herceptin] HERCEPTIN® Her-2/neu antibody (Fig. 18).

Please amend the specification on page 78, line 31 to page 79, line 3, as follows:

Small scale production runs were carried out with the two best ECD-PD and ECD-ΔPD clones. Cells were cultured in suspension under serum-free conditions for 120 hours at 33°C in the presence of 2 mM Sodium butyrate. The expression of both fusion proteins was evaluated by Western blot using the [Herceptin] HERCEPTIN® Her-2/neu antibody and by SDS-PAGE followed by silver staining using the Daiichi kit. Both fusion proteins were found to be expressed at +/- 100 µg/ml.

Please amend the specification on page 80, lines 6017, as follows:

The purified fusion proteins were analyzed by SDS-PAGE followed by silver staining using the Daiichi kit, and by Western blot, using the 8029K rabbit polyclonal antibody or the mouse [Herceptin] HERCEPTIN® Her-2/neu antibody. The analysis showed that the level of purity following the two purification steps was estimated at +/- 90% by densitometry (Biorad GS-700 Imaging Densitometer). The Western blot analysis showed that the monomers remained the major band all along the purification, that the level of oxydation was not

increased, and that the detection of the epitope of interest was not modified by the conditions of purification, as shown by using the [Herceptin] HERCEPTIN® Her-2/neu antibody. The total amount of each fusion protein recovered was measured using a colorimetric protein assay (DOC TCA BCA). This assays estimated that 2 and 4 mg of ECD-PD and ECD-ΔPD fusion protein, respectively, were purified from 75 ml of culture, with a level of purity of +/- 90%.

IN THE CLAIMS

93. (once amended) An isolated nucleic acid encoding a fusion protein comprising a HER-2/neu extracellular domain fused to a HER-2/neu phosphorylation domain, wherein the nucleic acid hybridizes under stringent conditions to the complement of a nucleic acid sequence encoding [protein has a sequence at least 80% identical to] the amino acid sequence of SEQ ID NO:6, wherein the hybridization reaction is incubated in a solution comprising 5x SSC at a temperature of 50-65°C and washed in a solution comprising 0.2x SSC and 0.1% SDS at a temperature of 65°C [or wherein the protein comprises a sequence at least 80% identical to the sequence of SEQ ID NO:3 fused to a sequence at least 80% identical to the sequence of SEQ ID NO:4], and wherein the protein is capable of producing an immune response in a warm-blooded animal.

94. (once amended) The nucleic acid of claim 93, wherein the nucleic acid encodes a fusion protein [comprises a] comprising an amino acid sequence [at least 80 % identical to the sequence] of SEQ ID NO:3 [fused] linked to an amino acid sequence [at least 80% identical to the sequence] inclusive of Gln 991 to Val 1256 of SEQ ID NO:2.

95. (once amended) The nucleic acid of claim 93, wherein the nucleic acid encodes a fusion protein [comprises a] comprising an amino acid sequence [at least 80% identical to the sequence] of SEQ ID NO:8 [fused] linked to [a] an amino acid sequence [at least 80% identical to the sequence] of SEQ ID NO:4.

96. (once amended) The nucleic acid of claim 93, wherein the nucleic acid encodes a fusion protein [comprises a] comprising an amino acid sequence [at least 80% identical to the sequence] of SEQ ID NO:8 [fused] linked to the amino acid sequence inclusive of Gln 991 to Val 1256 of SEQ ID NO:2.

97. (once amended) The nucleic acid of claim 93, wherein fusion protein comprises sequences that are linked via an amino acid linker.

98. (once amended) A viral vector comprising a [polynucleotide] nucleic acid [sequence] of claim 93.

99. (once amended) A [pharmaceutical] composition comprising the nucleic acid [molecule] of claim 93, and a [pharmaceutically] physiologically acceptable carrier or diluent.

100. (once amended) The [pharmaceutical] composition of claim 99, wherein the [pharmaceutical] composition is a vaccine.

101. (once amended) The [pharmaceutical] composition of claim 99, further comprising an immunostimulatory substance.

102. (once amended) The [pharmaceutical] composition of claim 99, wherein the nucleic acid [molecule] is a DNA molecule.

103. (once amended) An isolated nucleic acid encoding a fusion protein comprising a HER-2/neu extracellular domain fused to a fragment of the HER-2/neu phosphorylation domain, wherein the nucleic acid hybridizes under stringent conditions to the complement of a nucleic acid encoding [protein has a sequence at least 80% identical to] the amino acid sequence of SEQ ID NO:7, wherein the hybridization reaction is incubated in a

solution comprising 5x SSC at a temperature of 50-65°C and washed in a solution comprising 0.2x SSC and 0.1% SDS at a temperature of 65°C [or wherein the protein comprises a sequence at least 80% identical to the sequence of SEQ ID NO:3 fused to a sequence at least 80% identical to the sequence of SEQ ID NO:5], and wherein the protein is capable of producing an immune response in a warm-blooded animal.

104. (once amended) The nucleic acid of claim 103, wherein the nucleic acid encodes a fusion protein [comprises a] comprising an amino acid sequence [at least 80% identical to the sequence] of SEQ ID NO:3 [fused] linked to [a sequence at least 80% identical to] the amino acid sequence inclusive of Gln 991 to Arg 1049 of SEQ ID NO:2.

105. (once amended) The nucleic acid of claim 103, wherein the nucleic acid encodes a fusion protein [comprises a] comprising an amino acid sequence [at least 80% identical to the sequence] of SEQ ID NO:8 [fused] linked to [a] an amino acid sequence [at least 80% identical to the sequence] of SEQ ID NO:5.

106. (once amended) The nucleic acid of claim 103, wherein the nucleic acid encodes a fusion protein [comprises a] comprising an amino acid sequence [at least 80% identical to the sequence] of SEQ ID NO:8 [fused] linked to [a sequence at least 80% identical to] the amino acid sequence inclusive of Gln 991 to Arg 1049 of SEQ ID NO:2.

107. (once amended) The nucleic acid of claim 103, wherein fusion protein comprises sequences that are linked via an amino acid linker.

108. (once amended) A viral vector comprising a [polynucleotide sequence] nucleic acid of claim 103.

109. (once amended) A [pharmaceutical] composition comprising the nucleic acid [molecule] of claim 103, and a [pharmaceutically] physiologically acceptable carrier or diluent.

110. (once amended) The [pharmaceutical] composition of claim 109, wherein the [pharmaceutical] composition is a vaccine.

111. (once amended) The [pharmaceutical] composition of claim 109, further comprising an immunostimulatory substance.

112. (once amended) The [pharmaceutical] composition of claim 109, wherein the nucleic acid [molecule] is a DNA molecule.

113. (once amended) A method of making a fusion protein, the method comprising the steps of:

(a) introducing into a cell an expression vector comprising a [polynucleotide] nucleic acid according to claims 93 or 103;

(b) culturing the transfected cell; and

(c) purifying the expressed fusion protein.

116. (once amended) The method of claim 113, wherein the expressed fusion protein is purified by a two-step procedure, the procedure comprising:

(a) anion exchange chromatography [on Q sepharose High Performance Columns]; and

(b) hydrophobic chromatography [on Phenyl Sepharose 6 Fast Flow low substitution].

117. (new) The nucleic acid of claim 93, wherein the nucleic acid encodes a fusion protein comprising an amino acid sequence of SEQ ID NO:3 linked to an amino acid sequence of SEQ ID NO:4.

118. (new) The nucleic acid of claim 93, wherein the nucleic acid encodes a fusion protein comprising an amino acid sequence of SEQ ID NO:3 linked to an amino acid sequence of SEQ ID NO:5.

119. (new) The nucleic acid of claim 93, wherein the nucleic acid encodes an amino acid sequence of SEQ ID NO:6.

120. (new) The nucleic acid of claim 93, wherein the nucleic acid encodes an amino acid sequence of SEQ ID NO:7.

121 (new) The nucleic acid of claim 93, wherein the nucleic acid encodes a secreted fusion protein.

122. (new) The nucleic acid of claim 103, wherein the nucleic acid encodes a fusion protein comprising an amino acid sequence of SEQ ID NO:3 linked to an amino acid sequence of SEQ ID NO:5.

123. (new) The nucleic acid of claim 103, wherein the nucleic acid encodes an amino acid sequence of SEQ ID NO:7.

124. (new) The nucleic acid of claim 103, wherein the nucleic acid encodes a secreted fusion protein.